Anaerobic Biotransformation and Mobility of Pu and Pu-EDTA

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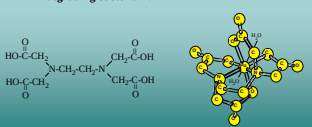
Summary

The complexation of radionuclides (e.g., plutonium (Pu) and ^{60}Co) by co-disposed ethylenediaminetetraacetate (EDTA) has enhanced their transport in sediments at DOE sites. Pu(IV)-EDTA is not stable in the presence of relatively soluble Fe(III) compounds. Since most DOE sites have Fe(III) containing sediments, Pu(IV) is likely not the mobile form of Pu-EDTA. The only other Pu-EDTA complex stable in groundwater relevant to DOE sites would be Pu(III)-EDTA, which only forms under anaerobic conditions. Research is therefore needed to investigate the biotransformation of Pu and Pu-EDTA under anaerobic conditions and the anaerobic biodegradation of Pu-EDTA. The biotransformation of Pu and Pu-EDTA under various anaerobic regimes is poorly understood including the reduction kinetics of Pu(IV) to Pu(III) from soluble (Pu(IV)-EDTA) and insoluble Pu(IV), the redox conditions required for this reduction, the strength of the Pu(III)-EDTA, how the Pu(III)-EDTA competes with other dominant anoxic soluble metals (e.g., Fe(II)), and the oxidation kinetics of Pu(III)-EDTA. Finally, soluble Pu(III)-EDTA under anaerobic conditions would require anaerobic degradation of the EDTA to limit Pu(III) transport. Anaerobic EDTA degrading microorganisms have never been isolated. Recent results have shown that *Shewanella oneidensis* MR-1, a dissimilatory metal reducing bacterium, can reduce Pu(IV) to Pu(III). The Pu(IV) was provided as insoluble PuO2. The highest rate of Pu(IV) reduction was with the addition of AQDS, an electron shuttle. Of the total amount of Pu solubilized (i.e., soluble through a 0.36 nm filter), approximately 70% was Pu(III). The amount of soluble Pu was between 4.8 and 3.2 micromolar at day 1 and 6, respectively, indicating rapid reduction. The micromolar Pu is significant since the drinking water limit for Pu is 10^{-12} M. On-going experiments are investigating the influence of EDTA on the rate of Pu reduction and the stability of the formed Pu(III). We have also begun to enrich and isolate bacteria capable of aerobic and anaerobic degradation of EDTA. Environmental samples (e.g., sludges, river sediments) were incubated aerobically and anaerobically with EDTA or NTA as the sole carbon and energy source. Aerobic enrichment with EDTA has not resulted in any cultures, but NTA has provided several isolates. Partial 16S rRNA gene sequence and sequence comparison identified four separate strains closely related to *Microbacterium oxydans*, *Aminobacter* sp., *Achromobacter* sp., *Aminobacter* sp., respectively. Anaerobic enrichments with either EDTA or NTA are still in progress since metabolism and growth is relatively slow. In addition to the biotransformation experiments, studies are underway to determine/validate complexation constants of Pu(III) with EDTA and the influence of competing ions on Pu(III)-EDTA complexes. These data are being obtained through solubility studies of $PuPO_4$(s) and $Pu(OH)_4$(s) as a function of time, pH, and EDTA and competing ion concentrations. These results have begun to fill-in knowledge gaps of how anaerobic conditions will influence Pu and Pu-EDTA fate and transport to assess, model, and design approaches to stop Pu transport in groundwater at DOE sites.

Introduction

EDTA (Figure below) can form strong water-soluble complexes with radionuclides and metals and has been used to decontaminate and process nuclear materials. EDTA was co-disposed with radionuclides (e.g., ^{60}Co, Pu) and has enhanced their transport in the subsurface. EDTA can also enhance the bioreduction of insoluble metals. There is poor understanding of how EDTA influences the bioreduction of Pu(IV) by *Shewanella oneidensis* MR-1. Three research areas are discussed in this poster. First, the speciation of Pu(III) and Pu(III)EDTA complexes. Second, the bioreduction of PuO_2(am) with and without AQDS and EDTA by dissimilatory metal reducing bacteria. Third, completion of past work on periplasmic binding protein for transport of EDTA into an EDTA degrading bacterium.



Task 1: Pu-EDTA Aqueous Chemistry

Research Objectives: Under anaerobic conditions Pu(III) is most likely the mobile species. Therefore, fundamental data for Pu(III) reactions, expected to be important in geologic environments are needed. We will determine the solubility of a sparingly soluble Pu(III) compound as a function of pH and [EDTA].

Hypothesis: EDTA will enhance the solubilization of Pu(III) in abiotic systems.

Approach: Solubility of $PuPO_4$(s) (a stable solid form of Pu(III)) was measured as a function of pH, phosphate, and time to develop/validate thermodynamic data for this system (Figs. 1 and 2).

Concentrations of EDTA and phosphate were fixed vs. pH and time to verify/determine the equilibrium constants for aqueous Pu(III)-EDTA complexes (Fig. 3).

Pu was maintained as Pu(III) in all experiments using reducing agents which included 0.004 M reduced AQDS solutions (pH <6.0) and $Na_2S_2O_4$ (pH ≥6.0).

Solvent extraction and UV-Vis-NIR were used for oxidation state analyses. Solids were separated from solutions using membrane filters.

Conclusion: A preliminary model[1] was used to interpret solubility data. The results show that EDTA forms very strong complexes with Pu(III) and that $PuEDTA^-$ are the dominant Pu species in a large range of environmental interest. This study will yield/validate fundamental data for the solubility product of $PuPO_4$(s), and phosphate and EDTA complexes of Pu(III) produced biotically.

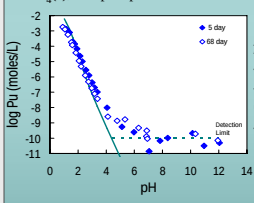


Figure 1. Solubility of $PuPO_4$(s) in 0.00032 M total phosphate as a function of pH. Steady state concentrations are reached in about 5 days. The dominant oxidation state of Pu is Pu(III) (not shown). Solid line = predicted [Pu] (see note[1]).

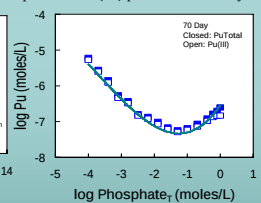


Figure 2. Solubility of $PuPO_4$(s) as a function of NaH_2PO_4 (pH = 2.5) at 70 days. Pu(III) is the dominant oxidation state of Pu in solution. Solid line = predicted [Pu] (see note[1]). Phosphate complexes of Pu(III) are weak and the increase in [Pu(III)] with the increase in $[PO_4]_T$ is primarily due to changes in ionic strength.

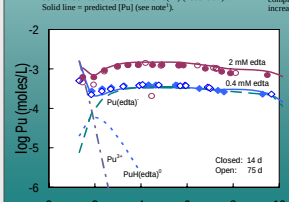


Figure 3. Solubility of $PuPO_4$(s) at fixed total concentrations of EDTA (2.0 or 0.4 mM) and total phosphate (0.00032 M) as a function of time and pH.

Equilibrium was reached rapidly and, in the presence of EDTA, $PuEDTA^-$ is the only important Pu(III) species in a large range of environmental interest.

Solid lines show total predicted concentrations and the other lines show predicted concentration of different species as marked for the 0.4 mM EDTA set (see note[1]).

[1]Note – Thermodynamic Data used in this Study: ΔG_f °RT values of Pu^{3+} = -233.558, $PuOH^{2+}$ = -313.33, $PuEDTA^-$ = -226.097, $PuHEDTA(aq)$ = -230.334, $PuPO_4$(s) = -703.46, PO_4^{3-} = -413.676, $EDTA^{4-}$ = 53.927, $[Pu^{3+}]$ H_3PO_4 = 0.655; other ion interaction parameters and values as listed in Rai et al. (2001) and Rai et al. (2003).

Task 2: Reduction of Pu by Microorganisms

Research Objectives: To elucidated the mechanism and rates of Pu(IV) and Pu(IV)-EDTA reduction by metal-reducing bacteria and determine where the Pu is located (in solution, biosorbed, bioaccumulated).

Hypotheses (subtask): *S. oneidensis* strain MR-1 can reduce Pu(IV)O_2(am) under anaerobic conditions. The electron shuttle AQDS will enhance the rate and magnitude of Pu reduction. EDTA will also enhance rates and magnitudes of biological Pu solubilization and reduction by enhancing the solubilization of Pu(IV) and Pu(III).

Approach: (1) Measure anaerobic Pu reduction by *S. oneidensis* MR-1 with H_2 as the electron donor and 500 µM Pu(IV)O_2(am) as the electron acceptor, in the presence and absence of 100 µM AQDS, pH=7.

(2) Measure anaerobic Pu reduction by *S. oneidensis* MR-1 with H_2 as the electron donor, 500 µM Pu(IV)O_2(am) as the electron acceptor, in the combined presence and absence of both 100 µM AQDS and 500 µM EDTA pH=7.

Measure Pu(aq) and Pu(III), Pu(IV), and Pu(V,VI) in the aqueous phase at days 1 and 6 (Experiment 1) and day 2 (Experiment 2 – analyses ongoing)

Conclusions: *S. oneidensis* MR-1 reduced Pu(IV) to Pu(III) within 2 days of exposure.

AQDS, an electron shuttle, significantly increased reduction (solubilization) of Pu(IV).

In the biological system, EDTA significantly increased Pu(IV) reduction.

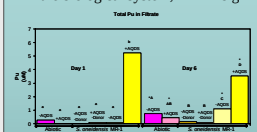
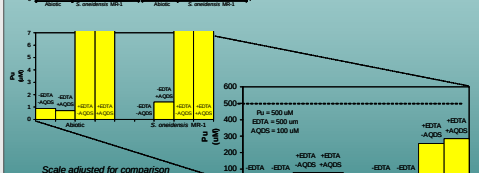


Figure 4. Recovery of Pu in the <0.36 nm fraction (aqueous). *S. oneidensis* with H_2 and AQDS significantly increases soluble Pu concentrations in the system; the addition of electron donor (H_2) alone did not greatly increase soluble Pu over the abiotic controls, however the addition of electron donor and an electron shuttle (AQDS) further significantly decreased the concentration of Pu(IV).



Scale adjusted for comparison with figure 1.

Figure 5. EDTA enhancement of Pu(IV) reduction after 2 days incubation. EDTA significantly increased the solubilization of Pu in the system,

(A) *S. oneidensis* MR-1 with EDTA further significantly increased the concentration of Pu in solution. While the enhanced solubilization of Pu by EDTA alone is difficult to ascribe to simple reduction, the further increase in solubilization by *S. oneidensis* MR-1 is almost certainly from the reduction of Pu(IV) to Pu(III).

(B) Graph 5A rescaled to the same y-axis as Figure 4.

This shows comparable microbial reduction of Pu(IV) in the abiotic and biotic –EDTA treatments of Figure 2A with the abiotic and biotic treatments shown in Figure 4 (abiotic +/-AQDS, and biotic +electron donor, +/-AQDS).

Task 3: Anaerobic Biodegradation of EDTA

Research Objective: Complete past work on the transport of EDTA into an EDTA degrading bacterium using the periplasmic binding protein, which confers specificity for transport by an ABC transporter.

Hypotheses: $MgEDTA^{2-}$ is the form of EDTA transported into the cell, which has to bind to the periplasmic binding protein before transport can occur.

Approach: Clone, express, and purify the periplasmic binding protein (EppA) from the EDTA degrading bacterium BNC1 using *E. coli*. Utilize various metal- and free-EDTA complexes to investigate binding of these substrates to EppA using isothermal titration calorimetry and fluorescence spectroscopy.

Conclusion: EppA bound free (i.e., no chelate metal) EDTA and NTA. $MgEDTA^{2-}$ likely dissociated before binding, while more stable complexes (e.g., $ZnEDTA^{2-}$) did not bind. Degradability of metal-EDTA is related to the formation of free EDTA to bind to EppA for transport into the cell. Results have been submitted for publication (Xun et al. 2006).

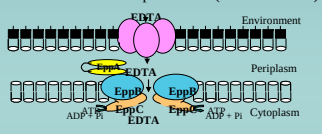


Figure 6. Free EDTA is transported into bacterial BNC1 cell by an ABC-type transporter for degradation to occur.

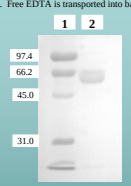


Figure 7. Purified EppA, the periplasmic binding protein

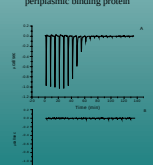


Figure 8. Calorimetric titration of EppA with Mg-EDTA (A) and Zn-EDTA (B).

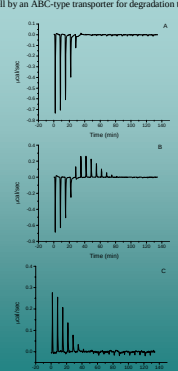


Figure 9. Calorimetric titration of EppA (A), EppA + Mg (B), and Mg (C) with EDTA.

Conclusions

1. **Pu(III) solubility data was collected and used to develop a model that demonstrated that $PuEDTA^-$ is the dominant species occurring within the range of environmental conditions modeled.**
2. ***S. oneidensis* MR-1 rapidly reduced Pu(IV) in dilute aqueous systems. This reduction was enhanced by the addition of electron donor (H_2) and electron shuttle (AQDS).**
3. **Degradability of metal-EDTA is related to the formation of free EDTA to bind to EppA for transport into the cell**

References

Rai, D., H. Bolton, Jr., B.A. Moore, N.J. Hess, and G.R. Choppin. 2001. Thermodynamic model for the solubility of PuO_2(am) in the aqueous Na+-H+-OH--Cl--H_2O ethylenediaminetetraacetate system. Radiochimica Acta 89:67-74.

Rai, D., A.R. Felmy, M. Yui. 2003. Thermodynamic model for the solubility of $PuPO_4$(c) in the aqueous Na+-H+-$H_2PO_4^-$-HPO_4^{2-}-OH--Cl--H_2O system. Journal of Radioanalytical and Nuclear Chemistry 256: 37-45.

Zhang, H., J. Dresman, H. Bolton, Jr., L. Xun. 2006. Characterization of a bacterial periplasmic binding protein of an ABC-type transporter for EDTA and NTA uptake. Submitted to Biochemistry, in review.

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